quantitative descriptions of DNA elasticity. Here we connect well-defined force-extension measurements with a novel structure-guided model for DNA elasticity, the twistable worm like chain model. This analytical description incorporates the essential physical characteristics of DNA, including how its helicity depends on extension. In addition, at forces exceeding ~65 pN, when DNA overstretches and melts, our experimental assay exposes rich features that can be fully attributed to the underlying base sequence. An equilibrium thermodynamic model is presented that quantitatively captures this melting behaviour solely based on the knowledge of DNA sequence and elasticity. These results offer a new standard description for the mechanics of DNA and enable deeper quantitative insight into the physical interactions of DNA-associated proteins.

400-Pos Board B200
Underwound DNA Under Tension: Structure, Elasticity and Sequence-Dependent Behavior
Maxim Sheinin, Scott Forth, John F. Marko, Michelle D. Wang.
Torque-induced separation of DNA strands plays an important role in a wide variety of cellular processes, such as transcription and replication. In the present work, we investigate mechanical properties of torsionally-melted DNA at the single-molecule level using an angular optical trap. While applying a constant tension to the DNA molecule, we simultaneously measure the extension change and torque as the DNA is being underwound up to and beyond the end of the melting phase transition. We find torsionally-melted DNA to be left-handed and flexible to bending, but with a relatively high torsional resilience. It is shown that our data can be incorporated into the recent DNA phase transition model. We have also discovered that at biologically relevant low forces, sequence has a significant impact on the underwound DNA properties. Implications of our findings for the global DNA force-torque phase diagram are discussed.

401-Pos Board B201
Sequence Dependent Structural Transitions in DNA Induced by Torque
Florian C. Oberstrass, Louis Fernandes, Zev Bryant.
B-DNA becomes unstable under superhelical stress and is able to adopt a wide range of alternative conformations including Z-DNA, cruciforms, and strand-separated DNA. Localized sequence-dependent destabilization of superhelical DNA is thought to be important for the regulation of biological processes requiring strand separation, such as transcription and replication initiation. To directly probe the effect of sequence on structural transitions driven by torque, we have measured the torsional response of a panel of DNA sequences using newly developed extensions of the rotor bead tracking assay[1]. In these assays, a submicron rotor bead is employed as a rotational probe attached to the side of a single stretched DNA molecule. Plots of torque as a function of twist show clear signatures of sequence-dependent cooperative structural transitions. Our results shed new light on the structure and stability of mismatched DNA sequences as well as the torsional properties of replication origins.
1. Bryant, Z. et al. *Nature* Vol. 424 338-41 (2003).

402-Pos Board B202
Insights Into Sequence Dependent Effects on DNA Elasticity Using Single Molecule Techniques
Krishnan Raghunathan, Yih-Fan Chen, Joshua Milstein, Benjamin Juliar, Justin Blaty, Jens-Christian Meiners.
The mechanical properties of DNA play an important role in regulating gene expression within a cell. Sequence does not only code, but also affects structural properties of the DNA. These variations can change the intrinsic curvature and the stiffness of the DNA. To study the effect of sequence on elasticity, it must be decoupled from curvature effects. For this aim, we designed two DNA constructs with similar curvature but different sequences and measured their elasticity in single-molecule stretching experiments with optical tweezers. We report substantial differences in their persistence length. We complement these experiments with studies on the effect of these differences in elasticity on protein-mediated DNA looping as a means of transcriptional control, using the Lac repressor as a model system.

403-Pos Board B203
Stretching Poly(A) to Investigate Elastic Behavior at Low Ionic Strength
Bennett S. Kalafut, Koen Visscher.
In addition to serving as the genetic template for protein synthesis in the ribosome, RNA folds in sequence-dependent ways to form structures that have regulatory or catalytic functions. Homopolymeric polyadenylic acid (poly(A))is known to form, due to base-stacking, single-stranded helices in solutions with ionic strength high enough to screen the self-repulsion of RNA's negatively charged backbone (e.g. 500 mM Na+), giving it elastic properties different than those of a random RNA sequence. Understanding the behavior of these helical sequences is necessary, for example, to in turn understand the folding of A-rich regions of riboswitches or the binding of poly(A) binding protein to mRNA's poly(A) tail.
When stretched using optical tweezers, well-screened poly(A) shows a clear helix-coil transition, manifested as a shoulder or plateau on the force-extension curve. By stretching poly(A) to sufficiently high force in low ionic strength solutions, we can probe the relative strengths of backbone repulsion and base stacking and moreover investigate the role of possible tight or diffuse binding of di- and polyvalent cations, thus furthering our understanding of helix formation in poly(A) sequences.
We have found that the helix-coil transition is less distinct at 100 mM Na+ than at 500 mM Na+ and totally absent at 10 mM Na+. These results and their implications, and additionally the effect of the divalent cation Mg++ on elasticity of poly(A), will be presented.

404-Pos Board B204
Probing DNA Stiffness with Magnetic Tweezers
Qing Shao, Sachin Goyal, Laura Finzi, **David D. Dunlap**.
The exceptional stiffness of DNA is routinely attributed to base stacking and repulsion between neighboring, negatively charged phosphates. Furthermore, well established biochemistry and recent single molecule experiments show that small, charged molecules and intercalating agents can dramatically alter the pitch and twist of the double helix. It is very likely that cells and macromolecular complexes have evolved to influence these phenomena in order to alter the flexibility of DNA to efficiently catalyze DNA transactions. In order to gain insight into how cells manipulate DNA efficiently, a quantitative understanding of DNA elasticity is necessary. Using magnetic tweezers to twist and stretch single DNA molecules, experiments were performed to probe the parameters of DNA stiffness. Although this technique is exquisitely sensitive and permits broad exploration of twist versus extension data, accurate models with which to interpret the stiffness parameters are critical. In particular, the knee point of the DNA extension versus twist curve, which is sensitive to both the bending and torsional rigidity of the molecule, is a signature, which if modeled accurately might give insight into how base stacking and electrostatics contribute to DNA stiffness. Our experiments showed that diaminopurine substitution for adenine, which adds an additional hydrogen bond to AT base pairs, stiffens DNA by about 50% without significantly changing the knee point. Instead adding low molecular weight polycations such as spermine or spermidine to the solution appeared to soften DNA and promote plectoneme formation at lower values of torsion. Thus base pair stability and, implicitly, stacking seem to have affected only the DNA elasticity while charge neutralization also favored the conversion of excess twist into writhe.

405-Pos Board B205
Synthesis and Single-Molecule Characterization of a DNA Hairpin Construct Based on the TAR RNA Sequence
Catherine A. Dietrich, Xiaowen Wang, Micah J. McCauley, Mark C. Williams, Megan E. Núñez.
Optical tweezers are used to examine the folding and unfolding of single molecules, revealing the effects of force, ionic strength, proteins, and small molecules on the kinetic and thermodynamic parameters of bimolecular stability. Here, we examine a DNA hairpin construct related to the trans-activation responsive region (TAR) RNA sequence of HIV-1. In an optical tweezers experiment, a bead at one end of a duplex DNA assembly is attached to a micropipette, while a bead at the other end is held in an optical trap, thus allowing investigators to relate picoNewton forces necessary to unfold the DNA with corresponding nanometer changes in length. Force extension/relaxation data are used to elucidate DNA folding and unfolding processes.
Our DNA construct design contains a central hairpin structure with long double-stranded "handles" attached to both the 3' and 5' end of the hairpin. The DNA ''handles'', each approximately 3 kb in length, were synthesized by PCR using biotin- and digoxigenin-labeled primers that serve to attach the final construct to 5 μm-diameter beads for use with the optical tweezers. The smaller central hairpin sequence was obtained via solid-phase organic synthesis. After purification and assembly with a linker oligonucleotide, the 5' handle and the hairpin structure were ligated together and purified by agarose gel electrophoresis. This product was then ligated to the 3' handle and again purified. Individual molecules of the final hairpin construct, approximately 6 kb in length, were bound to streptavidin or anti-digoxigenin-labeled beads and their unfolding behavior was studied using optical tweezers.

406-Pos Board B206
Single Molecule FRET Microscopy of Immobilized DNA Molecules: Keeping Track of Dye Integrity and Stoichiometry
Victoria Birkedal, Asger C. Krüger, Flemming Besenbacher.
Fluorescence Resonance Energy Transfer (FRET) at the single molecule level is a powerful technique for studying conformational changes of biomolecules. Dual color excitation schemes help sorting the single molecule data and quantifying FRET efficiencies within a single molecule. Here, we compare several data analysis methods for accurate FRET measurements and for discriminating

between conformational changes in single molecules and other effects caused by dye photophysics or multimers.
Single molecule FRET measurements were conducted on a series of immobilized double-stranded and G-quadruplex DNA molecules using a widefield microscope. Donor (Cy3/TMR) and acceptor (Cy5) molecules were both excited via an alternated laser excitation scheme. The double stranded DNA samples serve as molecular standards and G-quadruplex DNA structures, in this context, are interesting as they show large conformation diversity.

407-Pos Board B207
Twist/Writhe Partitioning in DNA Minicircles
Mehmet Sayar, Alkan Kabakcioglu, Baris Avsaroglu.
Here we present a systematic study of supercoil formation in DNA minicircles under varying linking number by using molecular dynamics simulations of a two-bead coarse-grained model. Our model is designed with the purpose of simulating long chains without sacrificing the characteristic structural properties of the DNA molecule, such as its helicity, backbone directionality and the presence of major and minor grooves. The model parameters are extracted directly from full-atomistic simulations of DNA oligomers via Boltzmann inversion, therefore our results can be interpreted as an extrapolation of those simulations to presently inaccessible chain lengths and simulation times. Using this model, we measure the twist/writhe partitioning in DNA minicircles, in particular its dependence on the chain length and excess linking number. We observe an asymmetric supercoiling transition consistent with experiments. Our results suggest that the fraction of the linking number absorbed as twist and writhe is nontrivially dependent on chain length and excess linking number. Beyond the supercoiling transition, chains of the order of one persistence length carry equal amounts of twist and writhe. For longer chains, an increasing fraction of the linking number is absorbed by the writhe.

408-Pos Board B208
Mechanical Analysis Methodology for DNA Minicircles Observed by Cryo-Em
Todd D. Lillian, Troy Lionberger, Davide Demurtas, Julien Dorier, Andrzej Stasiak, Edgar Meyhofer, Noel Perkins.
Recent cryo-electron microscopy (cryo-EM) images of DNA minicircles, about 100 basepairs in length, provide a new perspective on the mechanics of DNA. In essence, the resultant 3-dimensional reconstructions capture the mechanically deformed state of the double helix at an instant in time. Such deformations may include sites with high bending and/or torsional flexibility that could result from the tight bending required to cyclize, superhelical stress, and thermal fluctuations. Unfortunately, these reconstructions resolve only the DNA helical axis and provide no information about (i) how to register their known basepair sequence with the reconstruction and (ii) how torsional deformations are distributed along the length of the minicircle. In addition, the experimental procedures are complicated and consequently limit the the number of reconstructed minicircles to about 20. Our objective is to understand the mechanics of these DNA minicircles, and specifically, to develop a method capable of detecting the presence of kinks or torsional destabilizations in their cryo-EM reconstructions. To this end, we developed a modal analysis approach to describe the mechanics of DNA minicircles. In our method, we use the thermal modes of a homogeneous elastic rod representation for circular DNA to assign modal amplitudes to each reconstruction. The distribution of modal amplitudes for a population of minicircles provides a unique 'signature' dependent upon several variables, including superhelical density and the presence of kinks or torsional destabilizations. To test our method and predict these signatures, we developed a statistical mechanics model to simulate ensembles of DNA minicircles. This model can represent sequence dependence (elasticity/curvature) and prescribed kinks or torsional destabilizations. Our preliminary analysis suggests that the observed signatures are inconsistent with a homogeneous elastic rod and thereby implicate the role of sequence dependence, kinks or torsional instabilities.

409-Pos Board B209
Temperature Dependence of DNA Persistence Length
Stephanie Geggier, Alexander Kotlyar, Alexander Vologodskii.
We have determined the temperature dependence of DNA persistence length, a, using two different methods. The first approach was based on measuring the j-factors of short DNA fragments at various temperatures. Fitting the measured j-factors by the theoretical equation allowed us to obtain the values of a for temperatures between 5 and 42 C. The second approach was based on measuring the equilibrium distribution of the linking number between the strands of circular DNA at different temperatures. The major contribution into the distribution variance comes from the fluctuations of DNA writhe in the nicked circular molecules which are specified by the value of a. The computation-based analysis of the measured variances was used to obtain the values of a for temperatures up to 60°C. We found a good agreement between the results obtained by these two methods. Our data show that DNA persistence length strongly depends on temperature and accounting for this dependence is important in quantitative comparison between experimental results obtained at different temperatures.

410-Pos Board B210
Sequence Dependence of DNA Persistence Length
Stephanie Geggier, Alexander Vologodskii.
It is vital for many aspects of DNA-protein interaction to know how DNA bending rigidity depends on its sequence. In particular, this is important for understanding how nucleosomes position along DNA molecules. Although the problem has been discussed for decades, it remains unsolved. The main difficulty is that one has to measure the rigidity constants for DNA molecules of different sequences with very high accuracy. The only known method that provides the needed accuracy is based on cyclization of short DNA fragments. Here we used the method to find how DNA bending rigidity depends on its sequence. We addressed the problem using the dinucleotide approximation, in which particular values of rigidity constants are assigned to each of 10 distinct dinucleotide steps. We prepared DNA fragments, about 200 bp in length, with various quasi-periodic sequences, measured their cyclization efficiency, and fitted the data by a theoretical equation to obtain the bending rigidity constant (or persistence length). By combining the data for all fragments we were able to extract a full set of rigidity constants. To test the resulting set of constants we used it to design DNA sequences that should correspond to very low and very high values of a, prepared the corresponding fragments and determined their values of a experimentally. We obtained remarkably close agreement between the measured and calculated values of a, proving that we have found the correct solution of this long-standing problem. This result opens new opportunities to test different models of sequence specificity of DNA-protein interaction.

411-Pos Board B211
The Mean Looping Time of DNA
Assaf Amitai, Ivan Kupka, David Holcman.
In many gene expression systems, a protein located on the DNA can affect the expression of a gene far along the chain. It has been recognized that the DNA can form transient loops, bringing a specific region of the gene close to another. Thus, transcription can be activated when a transcription factor is positioned far away from its site. The frequency of bending is a characteristic time scale of the activation process.
The mean time for a DNA molecule to loop, bringing together two sites, is a fundamental factor that we studied. Various approximations have been used to model polymers. Interestingly, dsDNA has been found to be well described by the standard Rouse model, in which the polymer is described as a collection of bead monomers connected by harmonic springs. The Rouse model is relevant when the sites are at a distance considerably bigger than the DNA persistence length. When the distance between the sites is of several persistence lengths, the semi-flexible chain model is better suited to model the DNA dynamics. The polymer chain is subjected to random independent motion (Brownian motion). When the two monomers come closer than a certain distance, interaction takes place and the monomers connect. We assumed that the interaction rate is much faster than the encounter time, thus the process ends with the first encounter of the monomers. This allowed us to compute the asymptotic formula for the mean encounter time in the two models. We obtained precise estimates for this mean first encounter time in two and three dimensions. Brownian simulations confirm our formulas and we discuss consequences of our results for random gene activation in the nucleus.

412-Pos Board B212
The Effect of Monovalent Cations on the Thermal Stability of Small DNA Oligomers with Internal Loops
Nancy C. Stellwagen, Paul J. Barnard.
Although the effect of monovalent cations on the thermal stability of nucleic acid duplexes has been studied for many years, relatively little is known about cation effects on the stability of DNAs or RNAs with internal loops. We have therefore investigated the thermal stability of the DNA analog of the *let-7* microRNA::*lin-41* messenger RNA complex from *C. elegans*, which contains an asymmetric internal loop that kinks the helix backbone[1]. A DNA construct containing a symmetric internal loop at the same site was also studied, as well as a fully base-paired control. The melting temperatures of the two oligomers with internal loops are equal at low ionic strengths in solutions containing Na^+ ions. However, the melting temperature of the oligomer with the asymmetric loop is ~2°C higher in solutions with Na^+ ion concentrations greater than ~100 mM. The melting temperature of the oligomer with the asymmetric loop is also ~2°C higher than the oligomer with the symmetric loop when the solution contains K^+ ions. Binding studies indicate that both oligomers with internal loops, as well as the duplex control, bind Na^+ ions weakly, with an average